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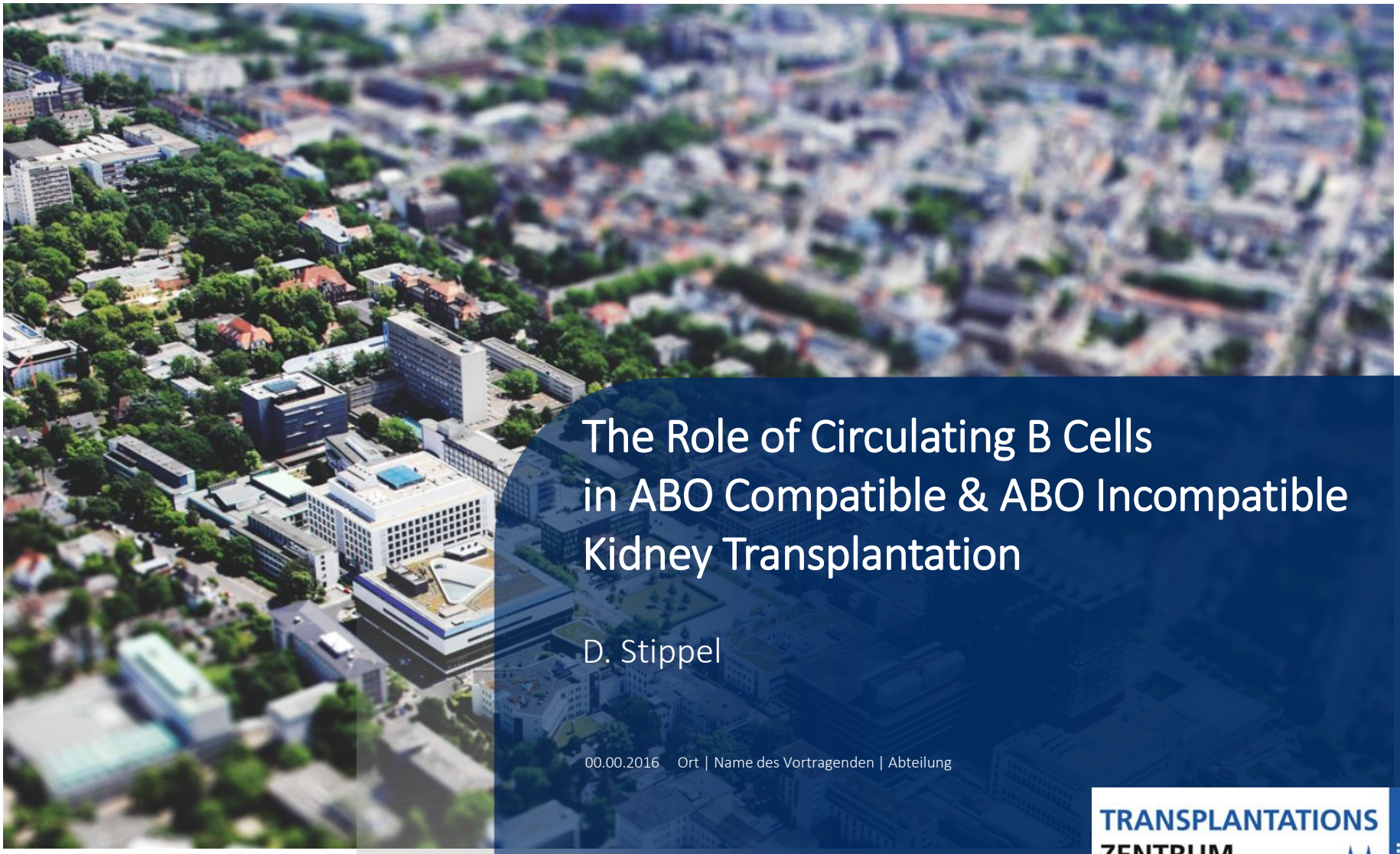
MANCHESTER INTERNATIONAL LIVING DONOR MEETING

Innovations and Controversies

23rd&24th April 2018

EKITA European Kidney Transplant Association



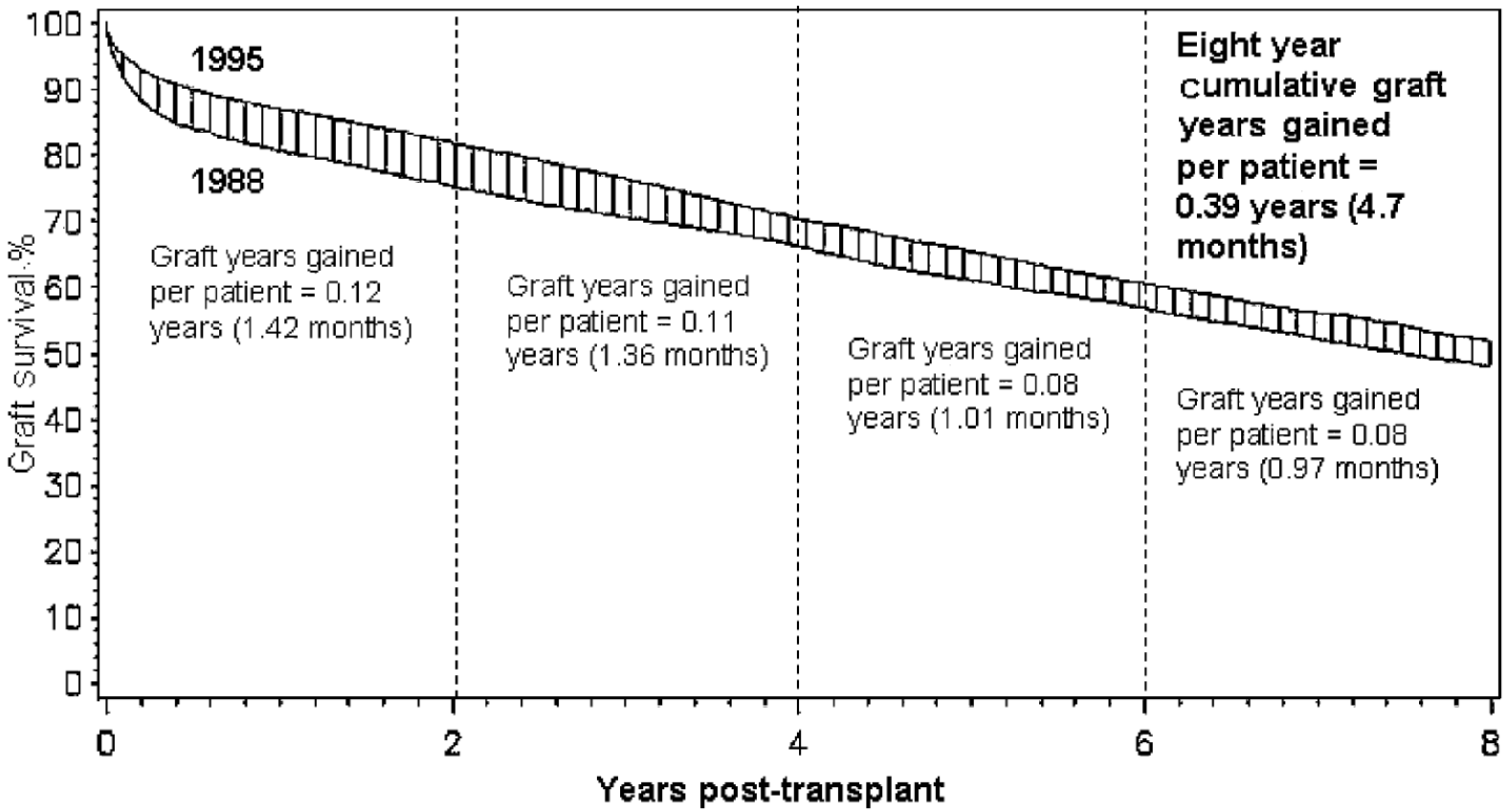
The Role of Circulating B Cells in ABO Compatible & ABO Incompatible Kidney Transplantation

D. Stippel

TRANSPLANTATIONS ZENTRUM KÖLN

Introduction

57961 deceased donation and 19976 living donation kidney transplants



Meier-Kriesche HU, AJT 2004

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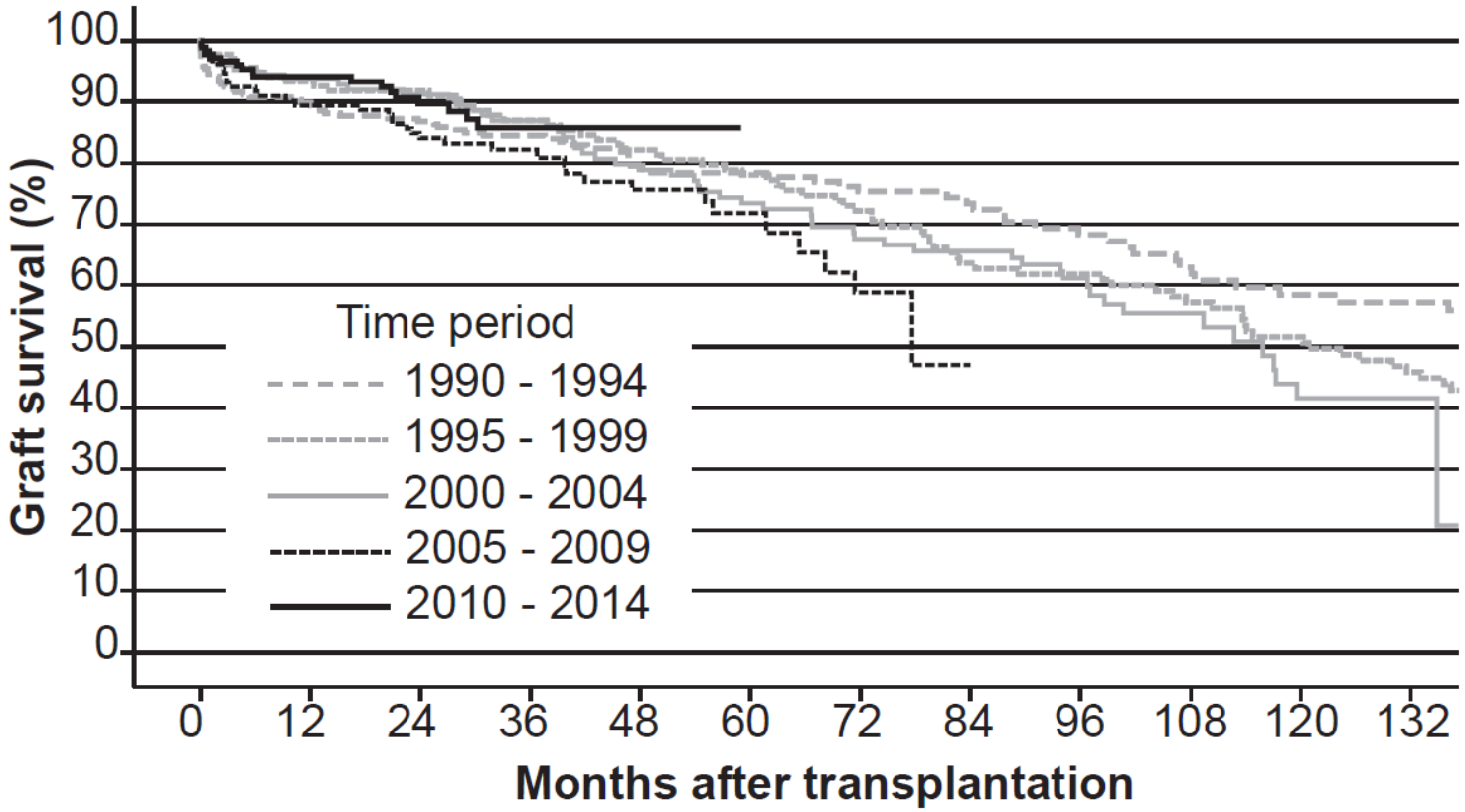
Situation in Cologne



- *Non immunological reasons*
- *Immunological reasons*

Impact of HLA antibodies

- patients with a stable transplant function
- follow up 2 years later

	Cytotoxicity	Flow	ELISA
No. tested	794	323	1573
Ab neg	627 (79.0%)	213 (65.9%)	1292 (82.1%)
Fail HLA -AK -	48 (7.6%)	11 (5.2%)	92 (7.1%)
Ab pos	167 (21.0%)	110 (34.1%)	281 (17.9%)
Fail HLA -AK +	30 (18.0%)	13 (11.8%)	35 (12.4%)
P value	0.00007	0.03	0.003

Terasaki, Transplantation 2005

Proof of principle

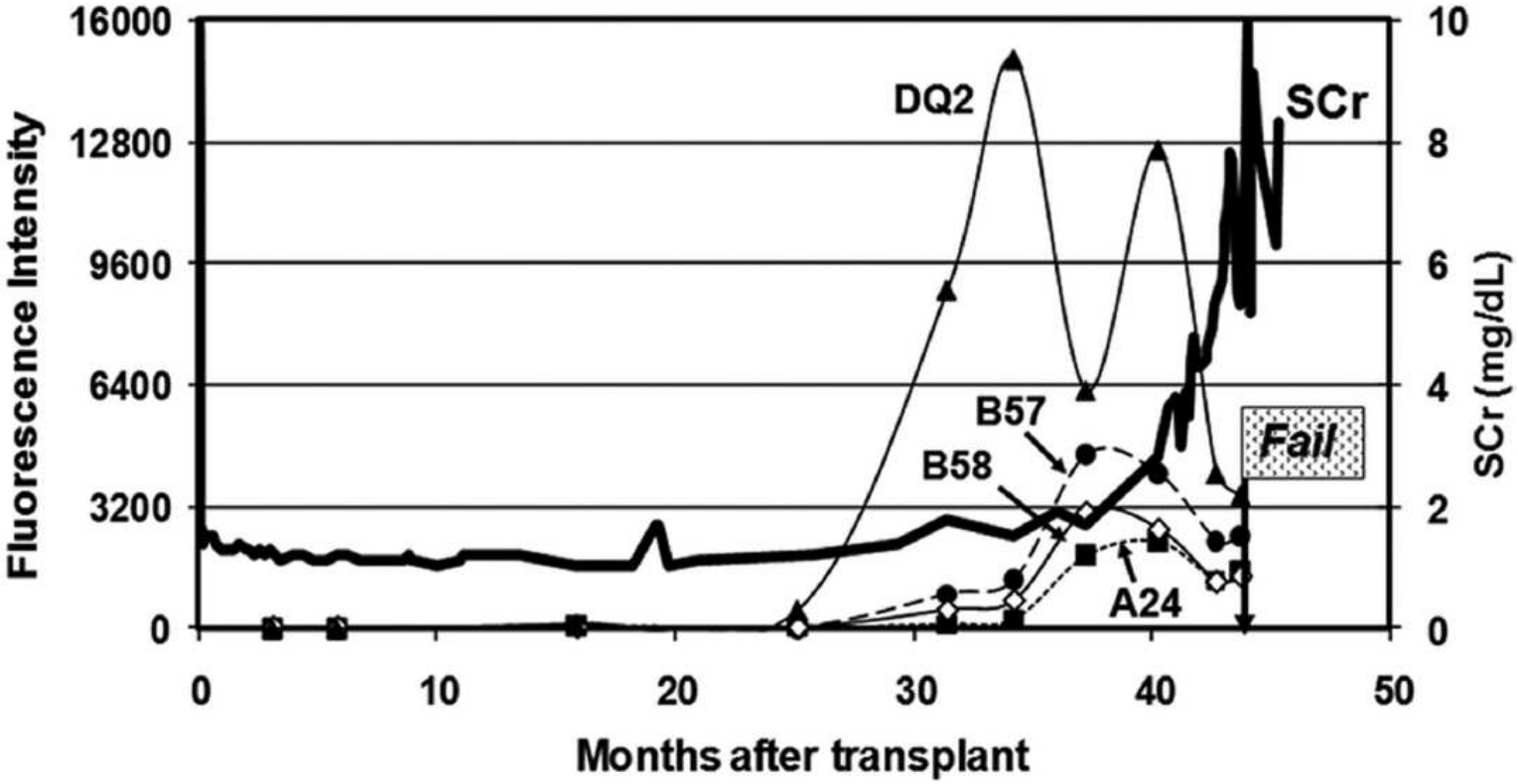
- documentation of time course

GF11

Patient: A30,-, B42,-, DR8,-, DQ7,-
Donor: A3,30, B42,58, DR7,8, DQ2,7

DSA: **B58**, DQ2

NDSA: A23,24, B49,53,**57,63**



Ozawa, Clin Transplantat 2006

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Antibody specificity

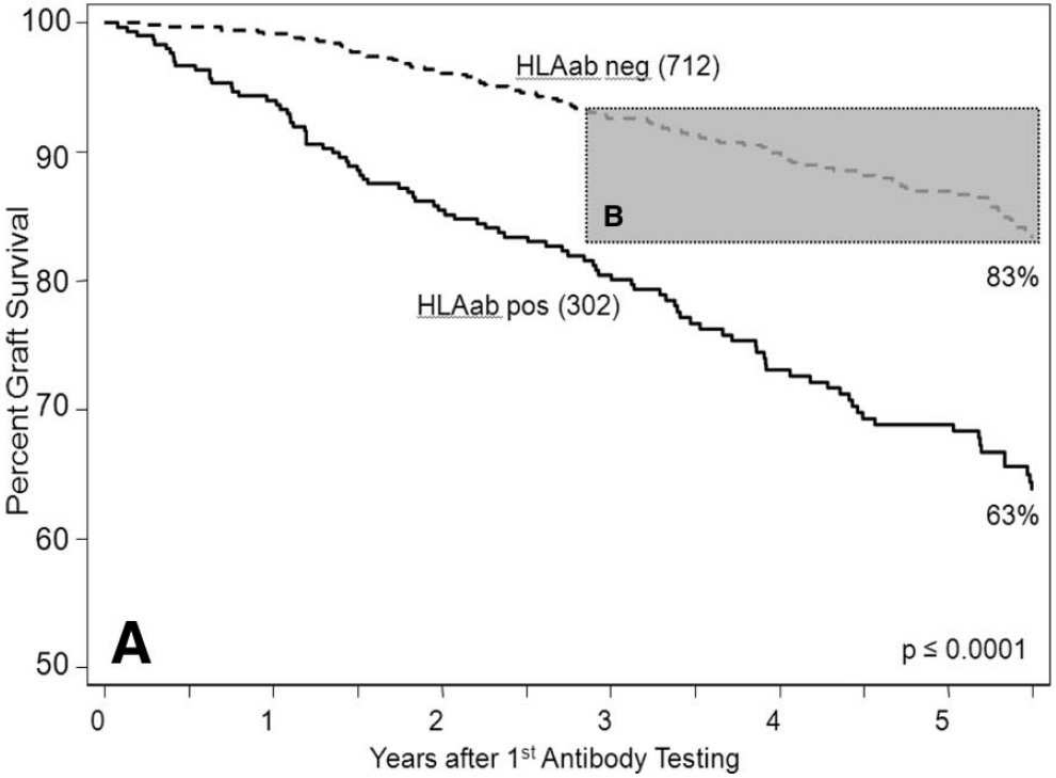
- HLA-AK versus DSA

1014 patients with stable renal function after transplant

on average 5 years after TX

Assay on HLA-Ab and DSA

Follow up for another 5 years



Lachmann, Transplantation 2009

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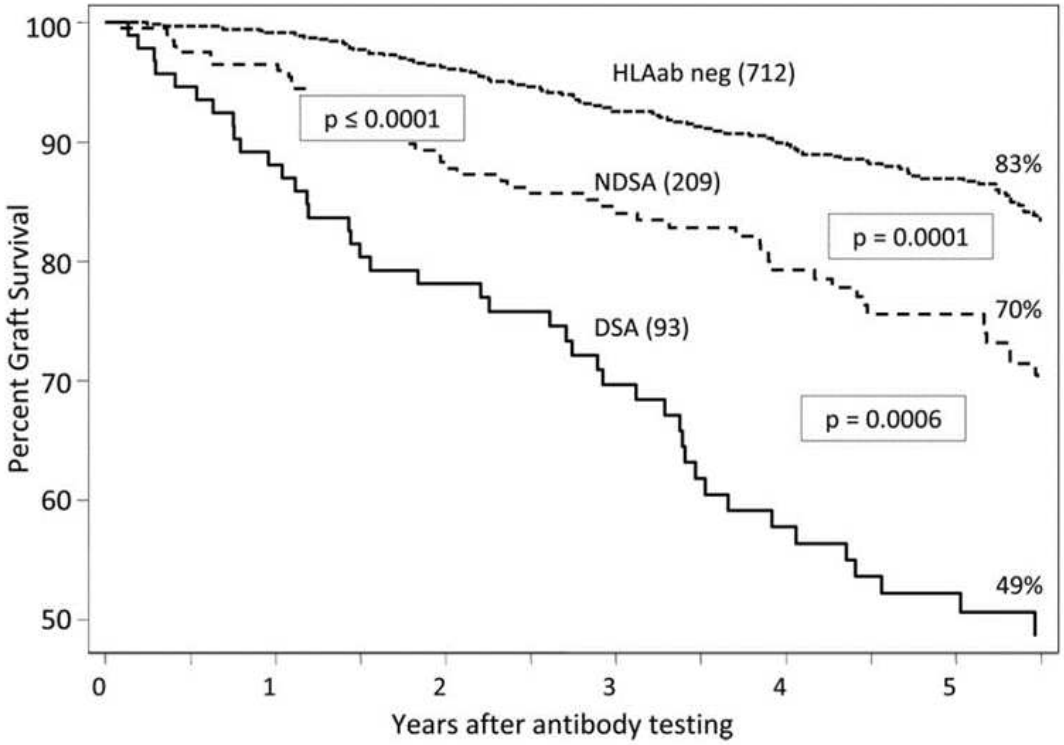

Lachmann, Transplantation 2009

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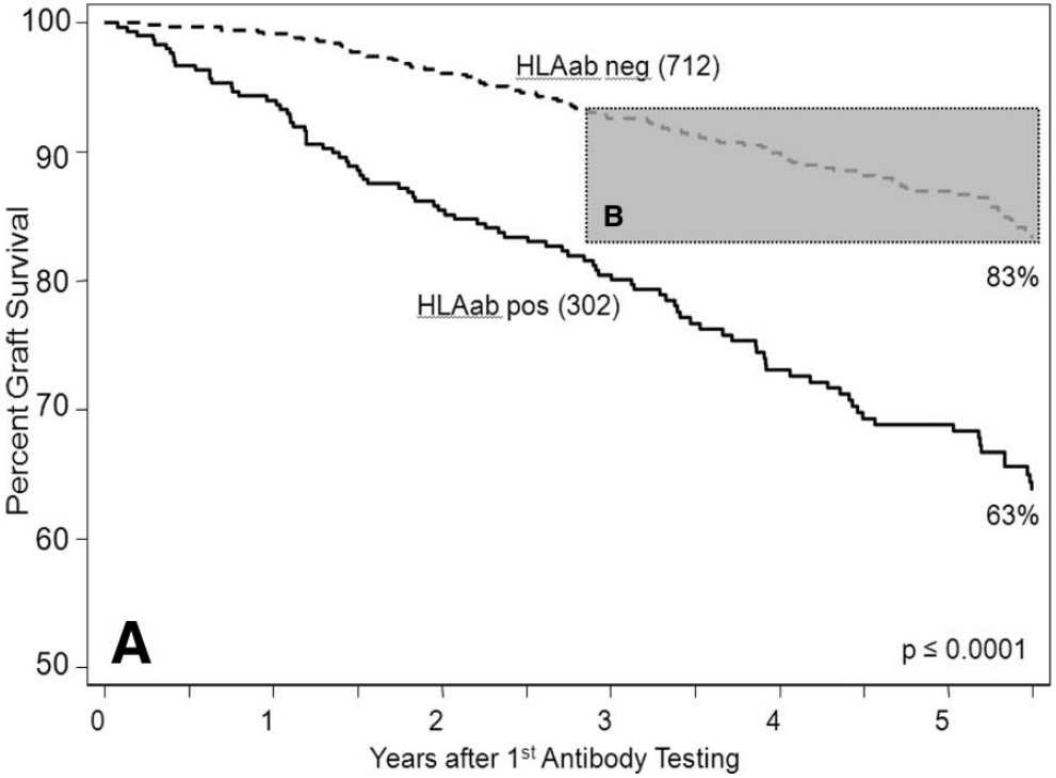

Lachmann, Transplantation 2009

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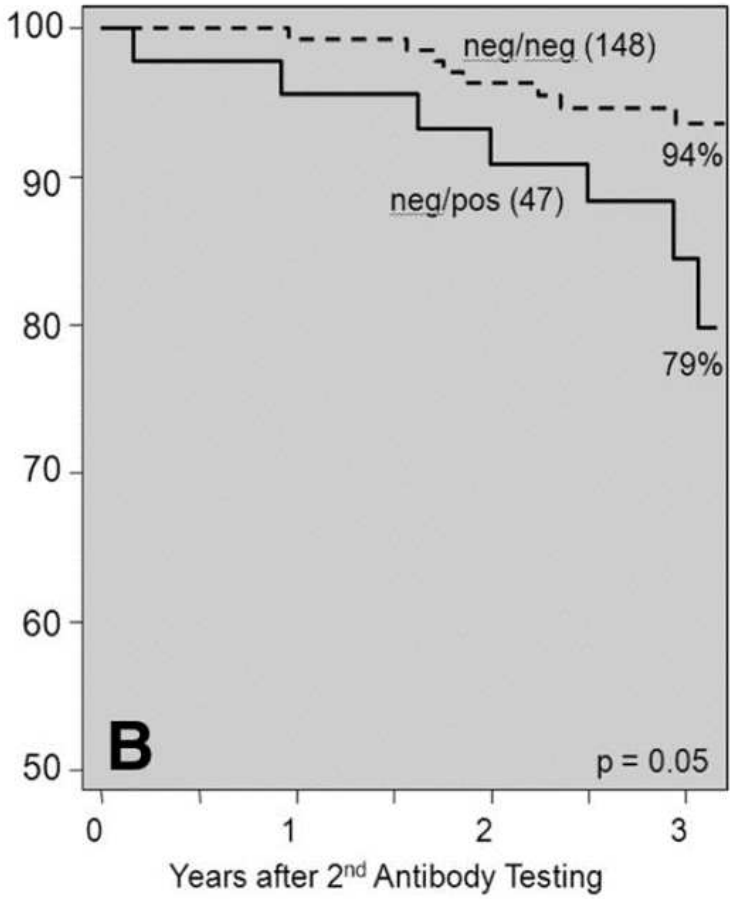

Lachmann, Transplantation 2009

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TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Impact of *de novo* donor-specific HLA antibodies detected by Luminex solid-phase assay after transplantation in a group of 88 consecutive living-donor renal transplantations

Georg Dieplinger,[1]* Vanessa Ditt,[2]* Wolfgang Arns,[3] Andrea Huppertz,[3] Tuelay Kisner,[4] Martin Hellmich,[5] Ursula Bauerfeind[2] and Dirk L. Stippel[1]

- 105 consecutive living donation NTX 1/08 – 10/11
  thereof 88 first-TX, no pretransplant HLA antibodies, no DSAs
- screening preTX using CDC, ELISA, Luminex
- Oligo-typing of HLA-A, -B, -C, -DR, -DQ locus
- postTX screening on de novo DSA (MFI > 100)
- follow up 659 ± 323 days (each > 1 year)
- multivariate analysis for AR and slope
- End points: 1 graft failure, 3 death with functioning graft

Occurrence of de novo DSA

- renal function

	All patients	DSA+	DSA-
creatinine (mg/dl) at day of discharge	1,43±0,71	1,47±0,71	1,41±0,71
Ø slope (mg/dl/year) first year	+0,03	+0,07	+0,01
Ø slope (mg/dl/year) after the first year	+0,06	+0,15	+0,02

Dieplinger, Ditt, Transplant Int 2014

Occurrence of de novo DSA

- rejection episodes

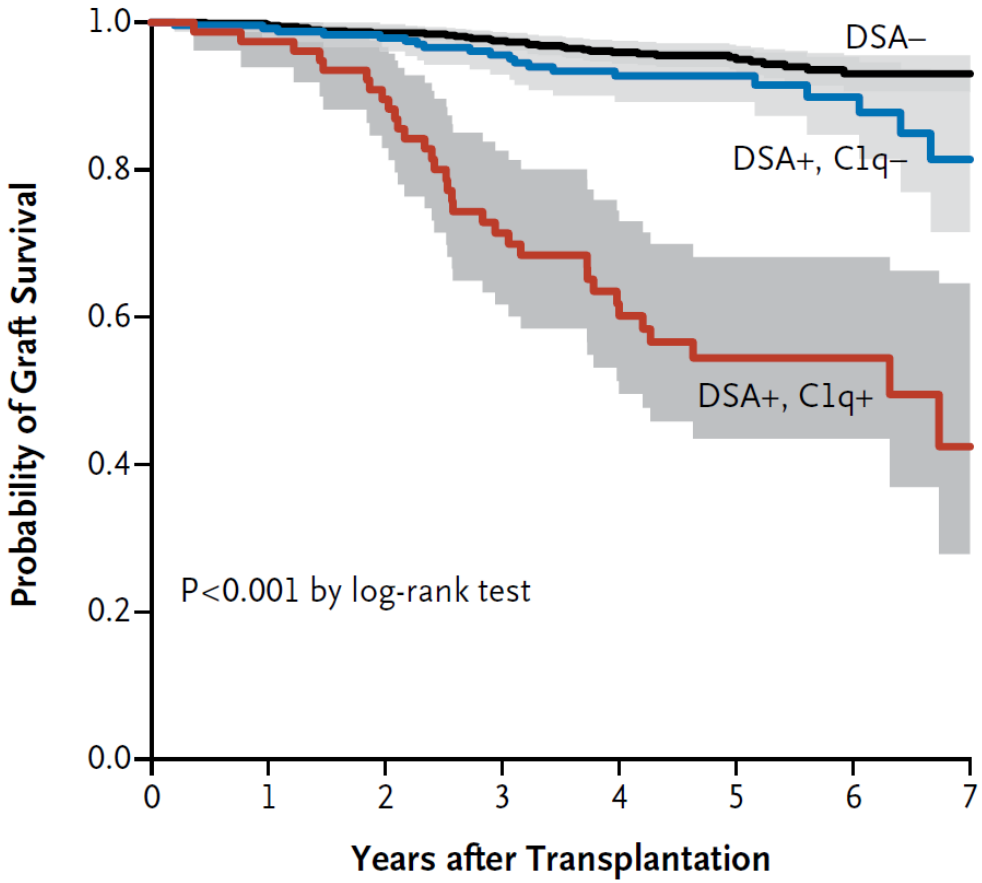
	All patients	DSA+	DSA-
Indication biopsy n (%)	44(50%)	17(55%)	24(44%)
AMR n(%)	5(6%)	4(13%)	1(2%)
TCMR	16(18%)	7(23%)	9(16%)

Dieplinger, Ditt, Transplant Int 2014

Are all DSA equally important?

- C1q bindende DSA

Kidney-Allograft Survival According to DSA and C1q Status



No. at Risk								
DSA–	700	698	667	612	504	338	164	38
DSA+, C1q–	239	237	227	181	139	80	44	14
DSA+, C1q+	77	75	68	48	37	20	12	5

Loupy, NEJM 2013

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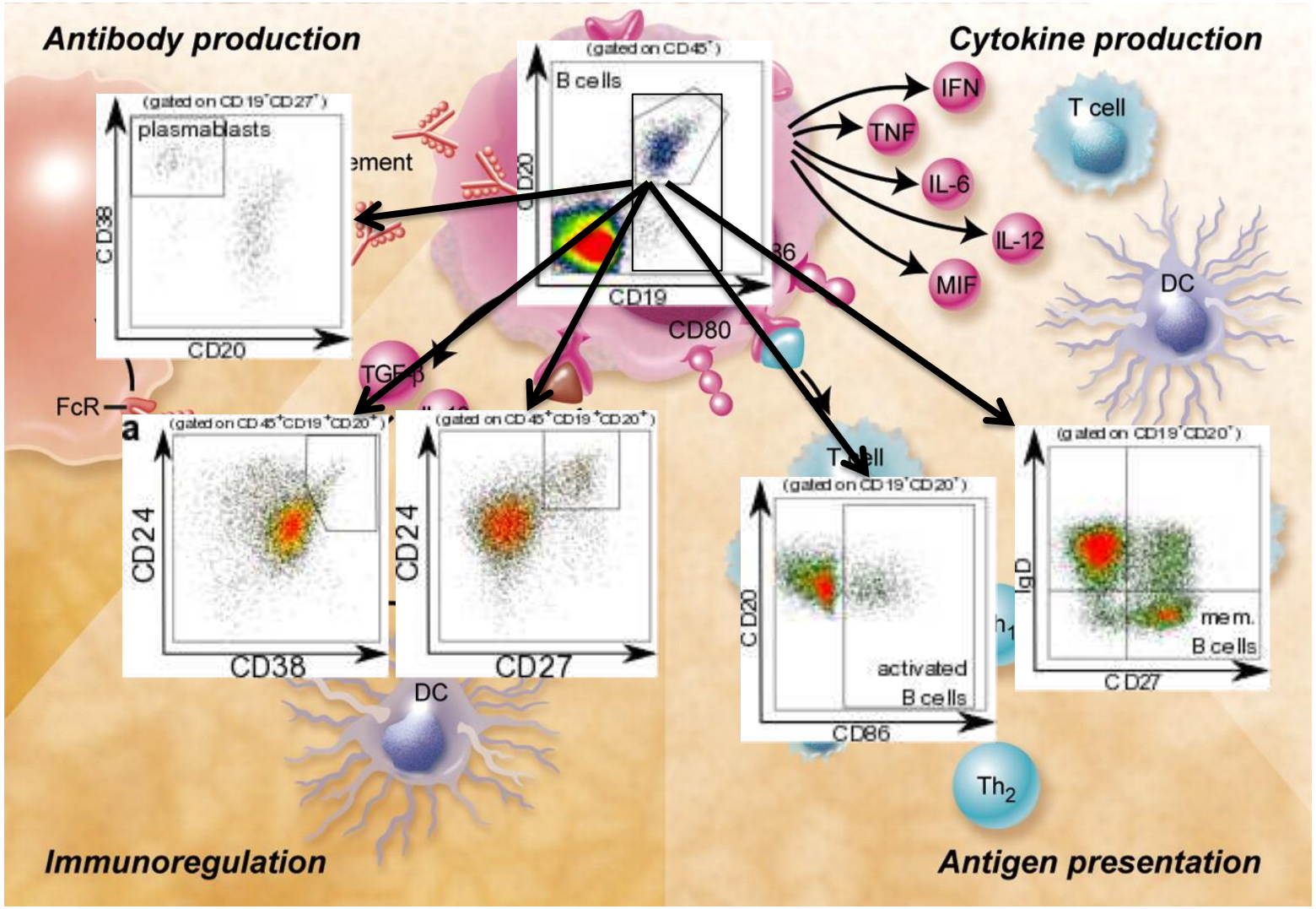
Functionally different B cell subsets

- more than antibody production



Shimabukuro-Vornhagen & Bergwellt Blood 2009

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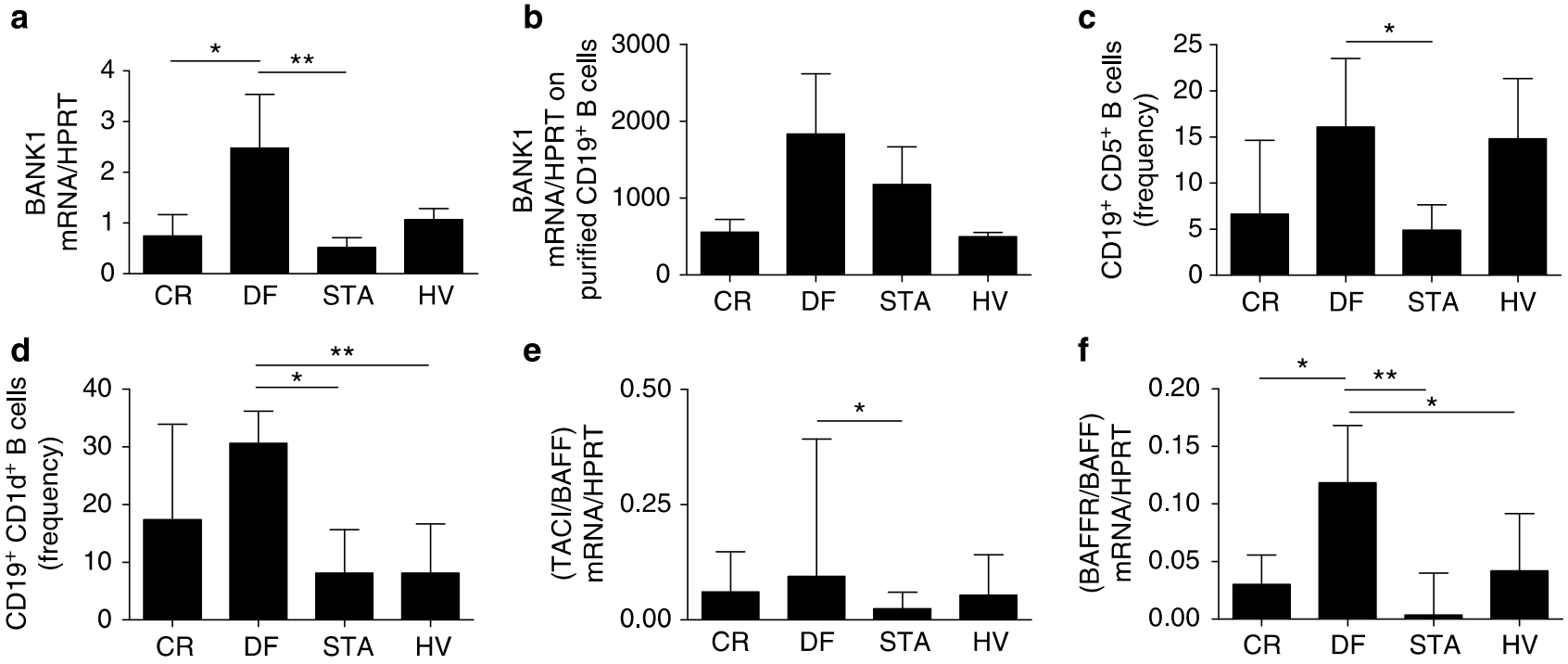
Introduction: B cell signature of tolerance



Pallier A, Kidney Int 2010

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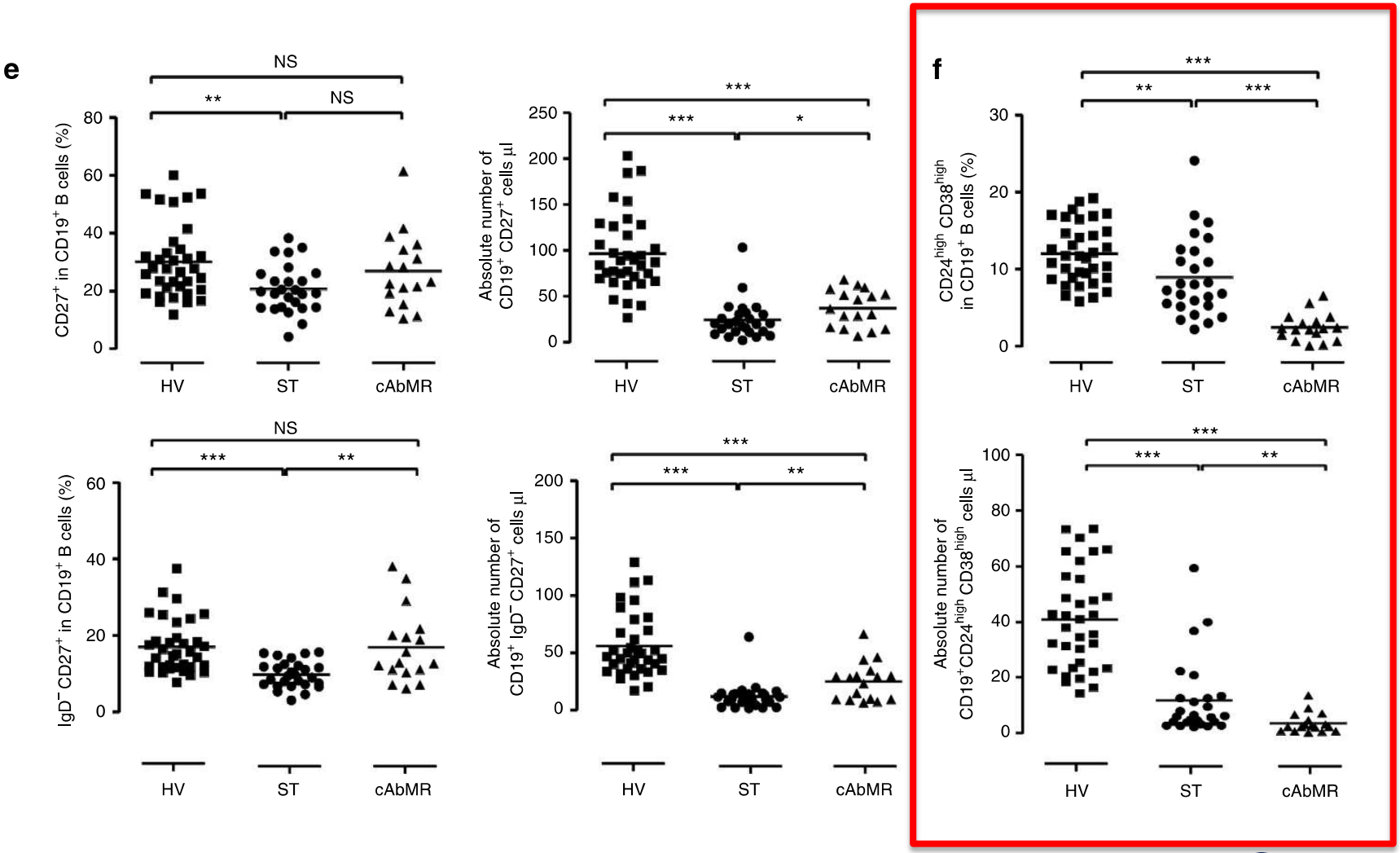
Introduction: Reduced regulatory B cells in cABMR



Nouel A, Kidney Int 2013

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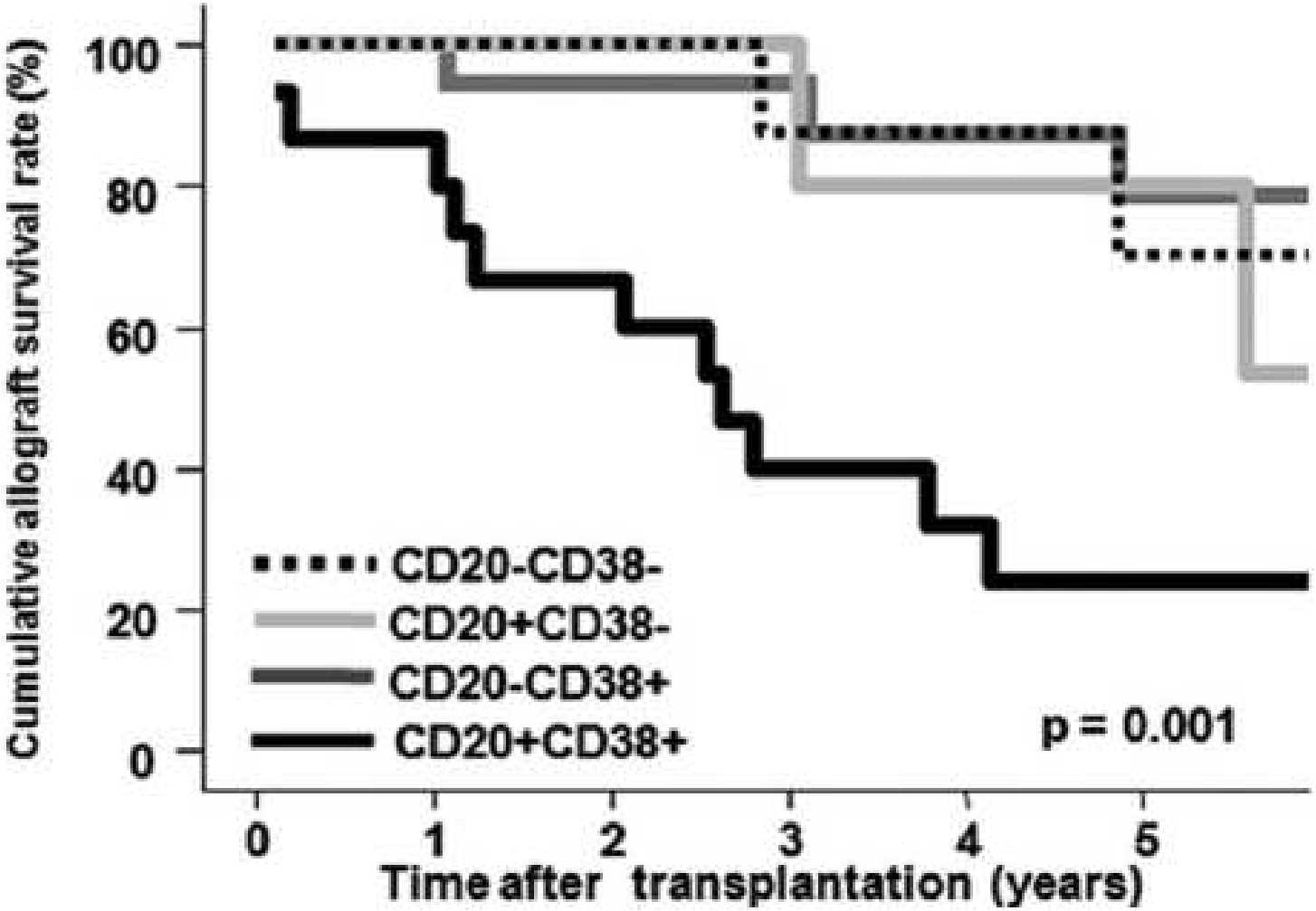
Introduction: Graft-infiltrating CD38$^+$ B cells impair graft outcome



Hwang HS, Transplantation 2010

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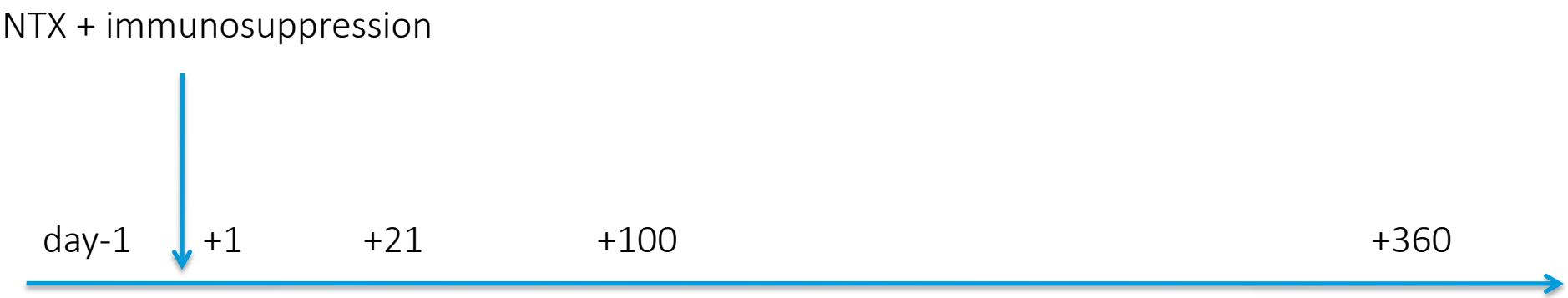
Study design: Flow cytometry of circulating B cell subsets

- Prospective, observational study
- AB0 compatible and AB0 incompatible transplants

  => pretransplant therapy with rituximab
- Retrospective analysis of severe rejections with graft loss



Schlößer H, AJT 2016

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Patient characteristics prospective cohort

Age		43.7(±15.7)
Sex	male	24 (68.5%)
	female	11 (31.5%)
Donortype	living-donor	30 (85.7%)
	deceased-donor	5 (14.3%)
Immunosuppression	Ciclosporin, Mycophenolic acid, Prednisone, Basiliximab	13 (37.1%)
	Tacrolimus, Mycophenolic acid, Prednisone, Basiliximab	12 (34.3%)
	Tacrolimus, Mycophenolic acid, Prednisone, Basiliximab, Rituximab	10 (28.6%)
Renal disease	Vesicoureteral reflux	3 (8.6%)
	Alport syndrome	3 (8.6%)
	Glomerulosclerosis	4 (11.4%)
	Polycystic kidney disease	5 (14.3%)
	Diabetic nephropathy	2 (5.7%)
	Glomerulonephritis	7 (20.0%)
	hypertensive nephropathy	2 (5.7%)
	atrophic kidney	3 (8.6%)
	others	6 (17.1%)
rejection before d365	yes	9 (25.7%)
	no	26 (74.3%)
HLA-Mismatch (±SD)		3.03 (±1.5)

Table 1: Demographic characteristics of prospectively included patients

Schlößer H, AJT 2016

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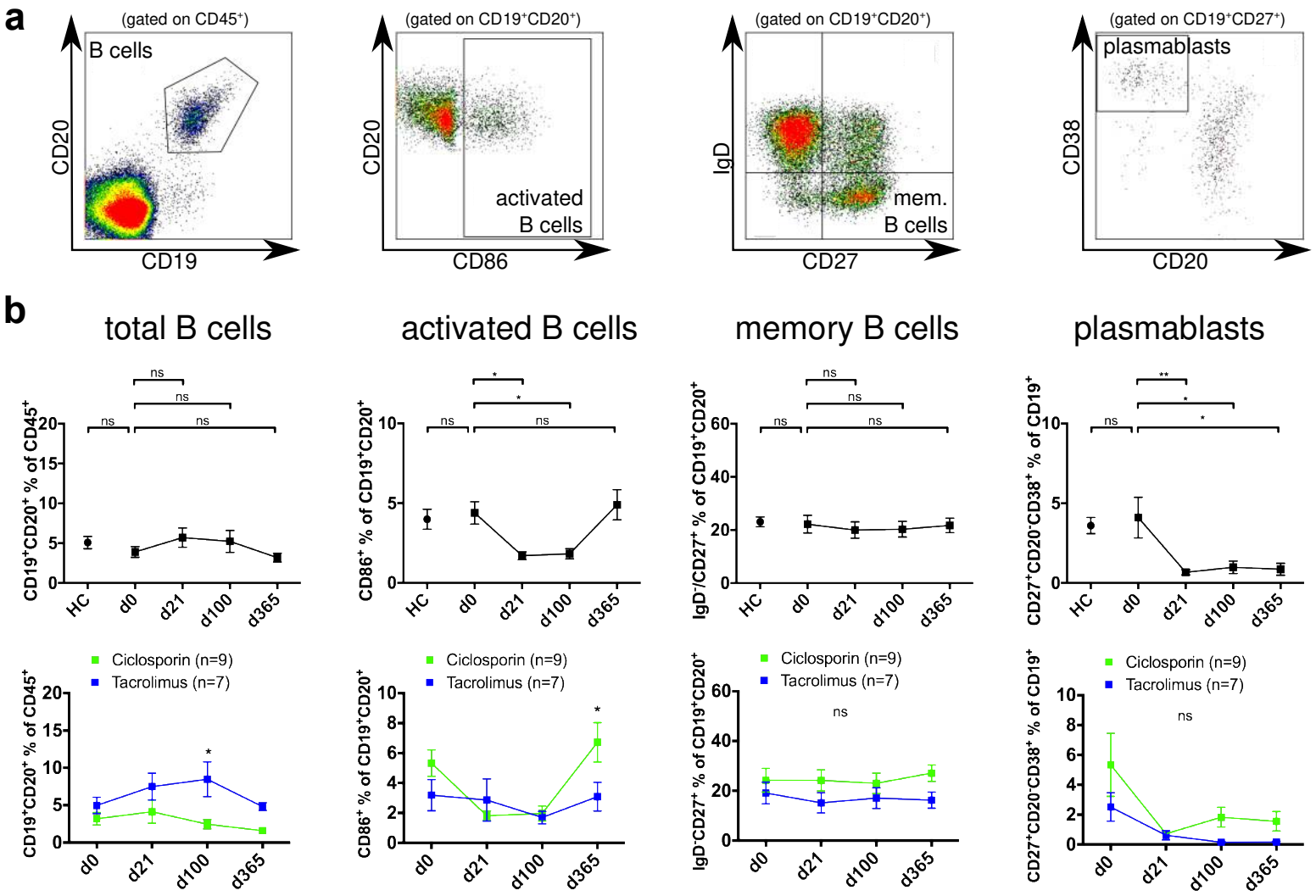
Evolution of effector B-cell subsets in stable patients



Schlößer H, AJT 2016

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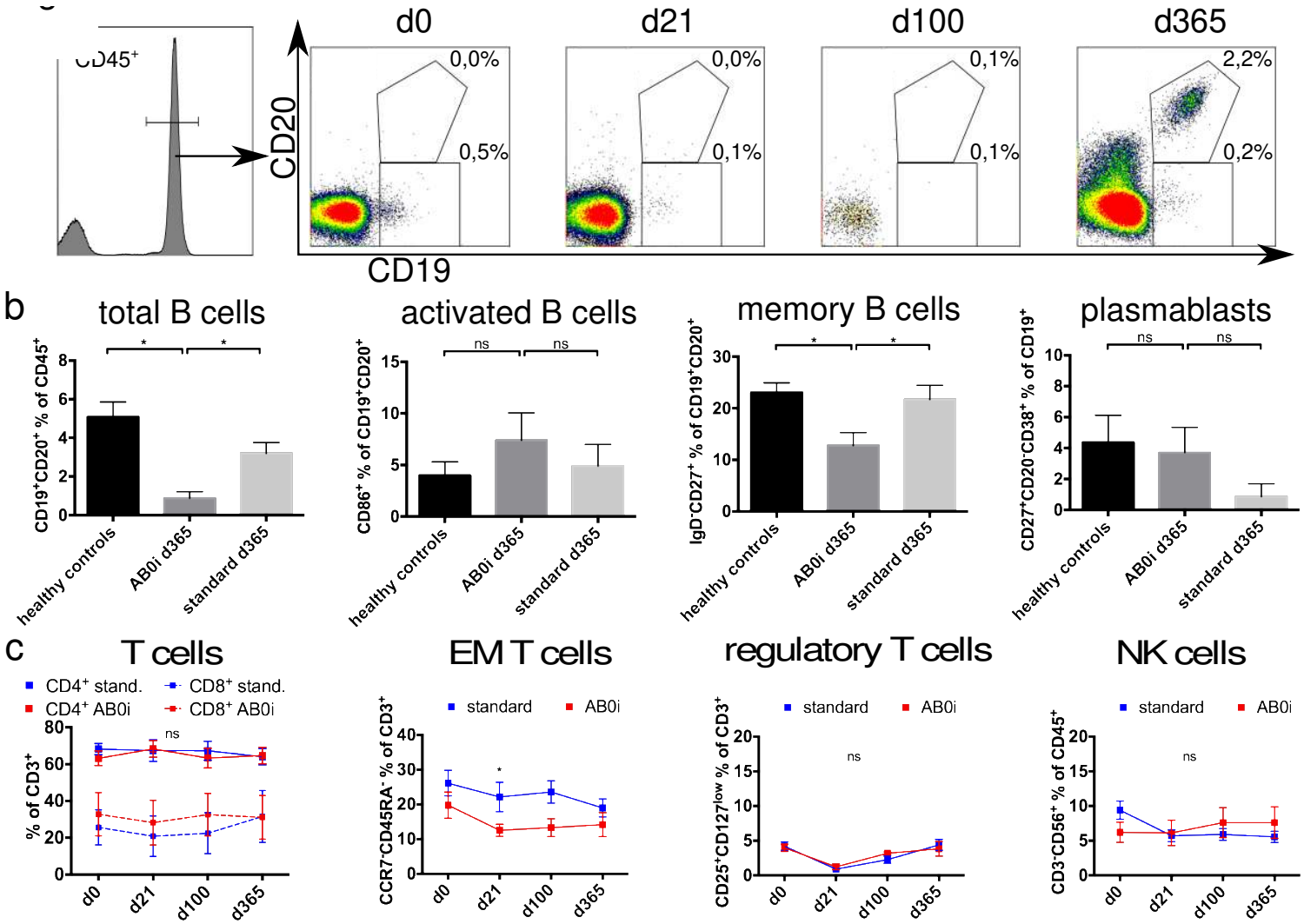
Evolution of T&B-cell subsets AB0i patients



Schlößer H, AJT 2016

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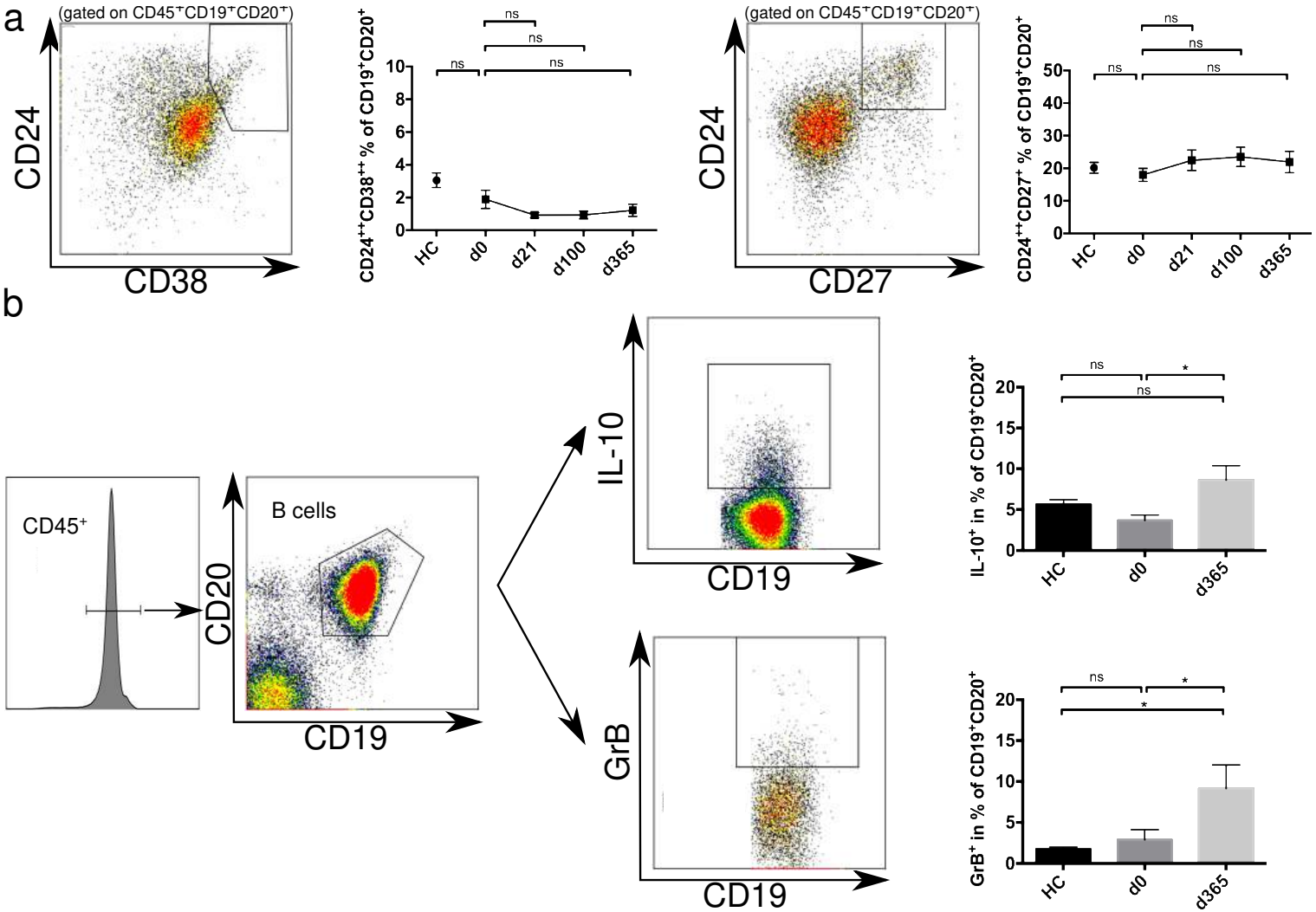
Evolution of regulatory B-cell subsets in stable patients



Schlößer H, AJT 2016

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Effector B cell subsets in allograft rejection

Samples		Banff 2013													DSA (HLA)	day post-Tx	type of rejection (treatment)
		i	t	v	g	ptc	C4d	ci	ct	cv	cg	mm	ah	ti			
Rejections in prospective cohort																	
	R1	2	1	0	0	0	0	3	3	1	0	0	0	3	no	179	borderline (prednisone)
	R2	0	1	0	0	0	0	0	0	0	0	0	0	0	no	60	borderline (prednisone)
	R3	2	1	0	0	0	0	1	1	3	0	0	0	2	no	51	borderline (prednisone)
	R4	3	1	0	2	2	3	3	3	2	2	0	1	3	yes (I+II)	30	ABMR (ATG+IA)
	R5	1	1	0	0	0	0	0	1	1	0	0	0	1	no	120	borderline (prednisone)
	R6	3	1	0	3	3	3	1	1	1	0	0	1	3	yes (I+II)	210	ABMR (ATG+IA)
	R7	3	1	1	2	0	0	0	0	0	0	0	0	3	no	8	TCMR IIA (ATG)
	R8	2	1	0	0	2	0	1	1	0	0	0	0	3	no	28	borderline (prednisone)
	R9	1	1	1	2	1	0	1	1	2	1	0	0	1	no	6	TCMR IIA (prednisone)
Acute rejections prior to treatment																	
	A1	3	2	2	3	0	3	3	3	3	1	0	0	3	yes (I+II)	574	TCMR (IIB) and ABMR
	A2	0	0	1	3	0	x	3	3	3	3	0	1	3	n.d.	1268	TCMR (IIA) and ABMR
	A3	2	1	1	2	0	0	0	0	0	0	0	1	2	yes (I)	10	ABMR
	A4	2	1	0	0	0	0	3	3	1	0	0	0	3	no	180	borderline
	A5	1	1	0	0	0	0	0	1	0	0	0	0	0	no	12	borderline
	A6	1	1	0	0	0	0	1	1	1	0	0	1	1	no	21	borderline
	A7	1	1	0	0	0	0	0	0	0	0	0	0	0	no	21	borderline
	A8	1	1	x	x	0	0	0	1	x	x	x	x	2	no	2977	borderline
	A9	0	0	0	0	0	0	3	3	2	0	0	1	1	yes (I)	2575	ABMR
	A10	2	2	0	0	0	0	1	1	0	0	0	0	2	no	143	TCMR (IA)
	A11	2	1	0	0	0	0	1	1	0	0	0	2	2	no	2176	borderline

Schlößer H, AJT 2016

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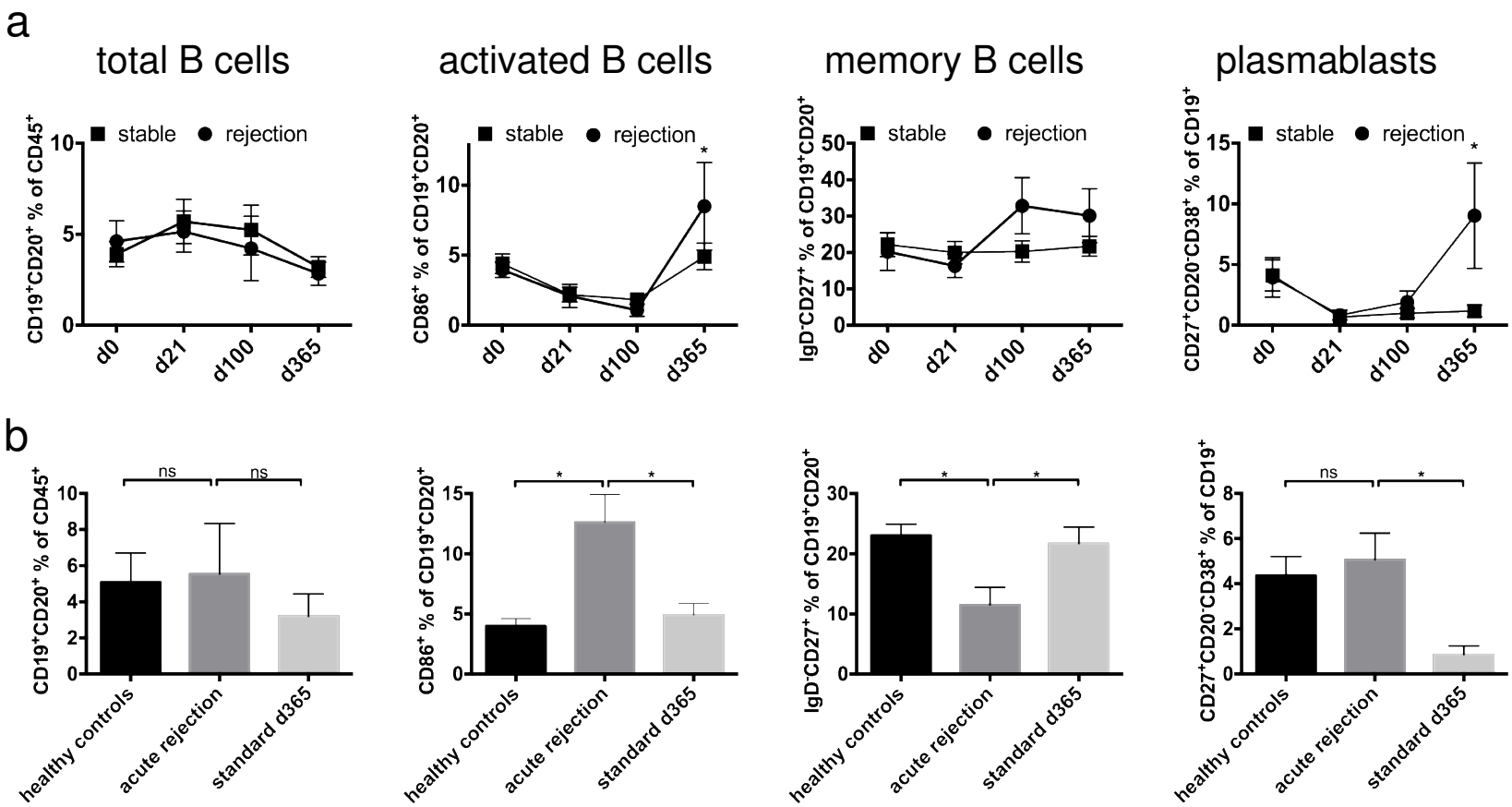
Effector B cell subsets in allograft rejection



Schlößer H, AJT 2016

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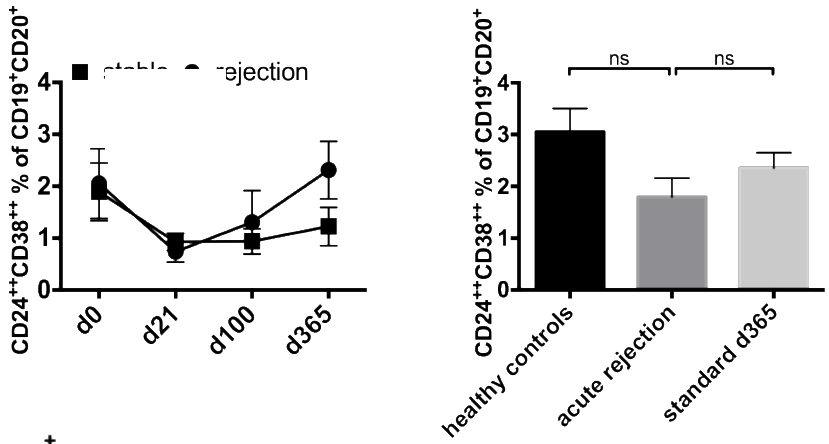
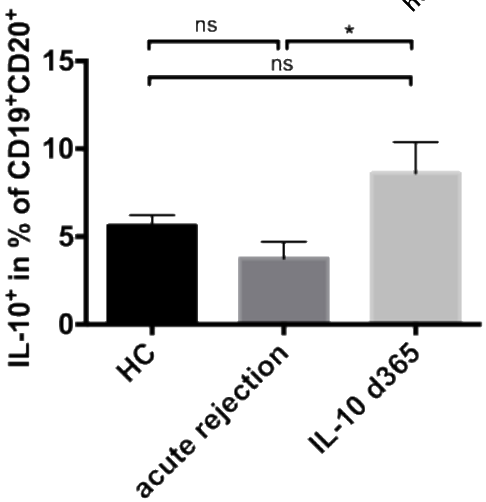
Regulatory B cell subsets in allograft rejection

IL-10

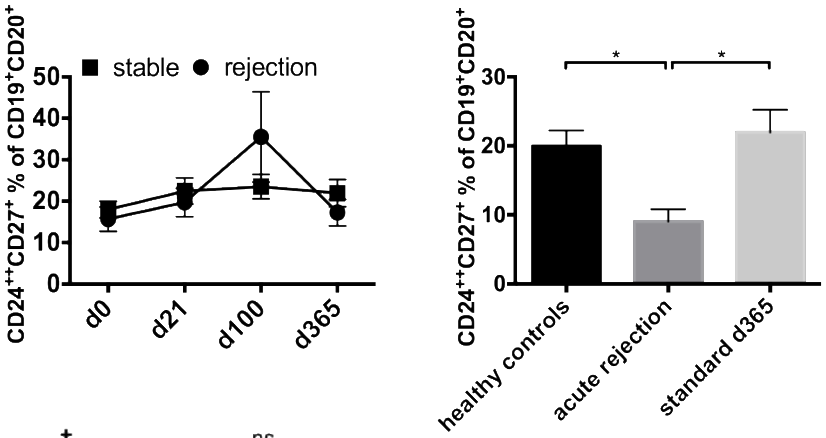
$CD24^{high}CD38^{high}$ B cells

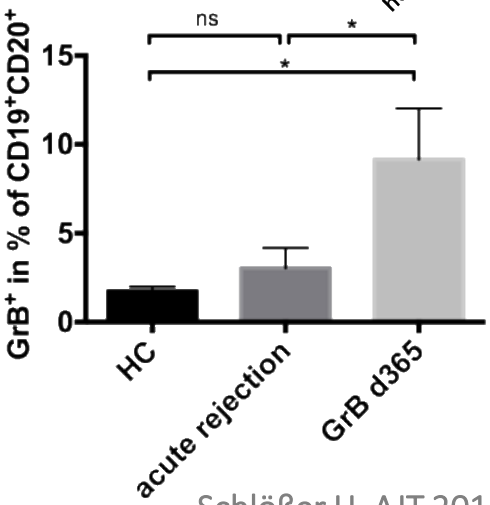




Granzyme B

$CD24^{high}CD27^{+}$ B cells





Schlößer H, AJT 2016

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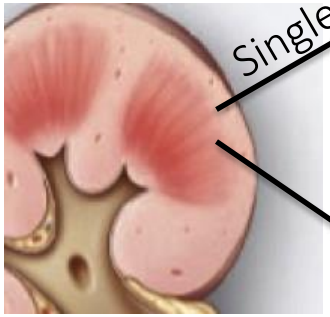
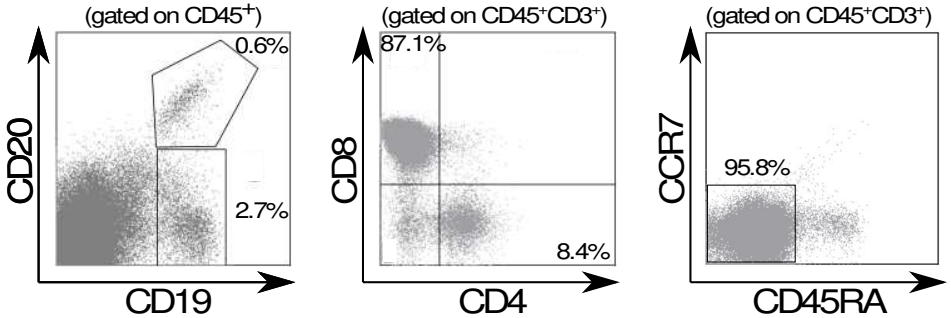
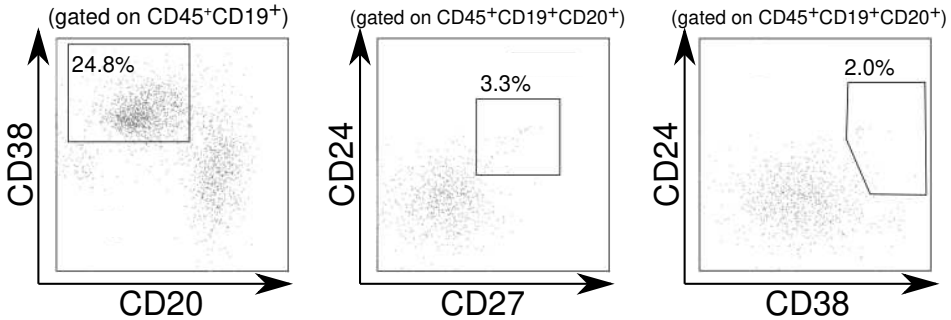
Graft-infiltrating B cells

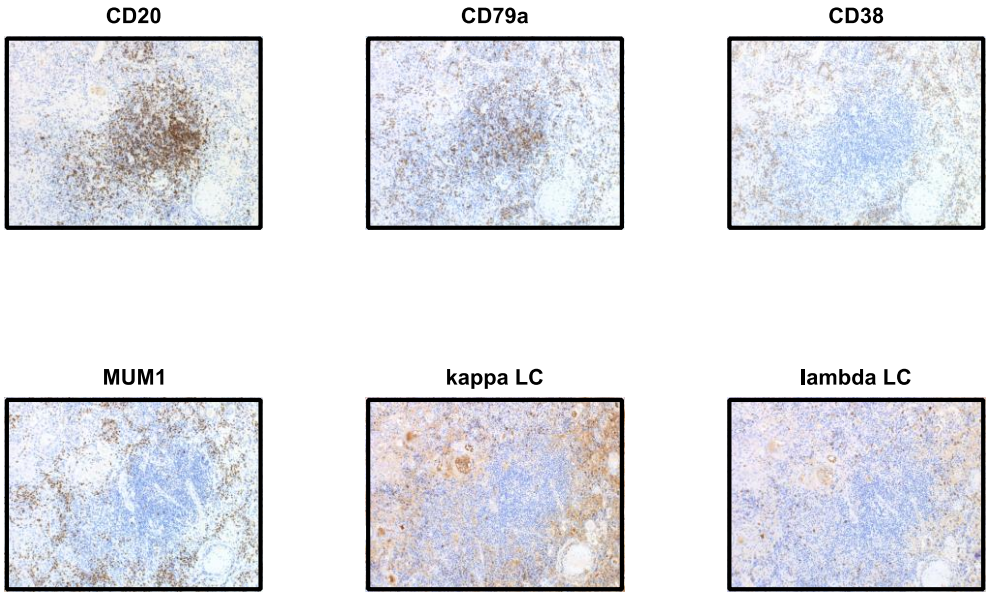


Single cell suspension

IHC







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Blood biomarkers of kidney transplant rejection, an endless search?

Biomarker	Measure	Timing	Clinical context
TEMRA $CD8^+$ T cells	Cell frequency	Pre/Posttransplantation	Acute rejection/kidney graft failure
IFN-γ ELISPOT	Cell frequency	Pre/Posttransplantation	Acute rejection/kidney graft function
Immunoknow	Intracellular ATP concentration	Posttransplantation	Acute rejection
Perforin	mRNA	Posttransplantation	Acute rejection
Granzyme B	mRNA	Posttransplantation	Acute rejection
FoxP3	Cell frequency, mRNA	Posttransplantation	Acute rejection /Chronic rejection
TSDR of FoxP3 gene	% of demethylation	Posttransplantation	Operational tolerance
B cell phenotype	Cell frequency	Posttransplantation	Acute rejection/cABMR
NK subset redistribution	Cell frequency	Posttransplantation	Anti-HLA immunization
kSORT	mRNA	Posttransplantation	Acute rejection/subclinical rejection
TruGraf™	mRNA	Posttransplantation	Acute rejection
miRNA profiles	miRNA	Posttransplantation	CAMR/TCMR
Donor-derived cell-free DNA	Cell-free DNA	Posttransplantation	Acute rejection

Jaquemont L, Expert Review of Molecular Diagnostics 2017

Conclusions (1)

- triple immunosuppression plus basiliximab has a remarkable effect on circulating B-cell subsets in renal transplant recipients

- significant differences between stable and rejecting patients concerning activated B-cells and plasmablasts

- Rituximab treatment resulted in AB0i patients in a long-lasting B-cell depletion and a naive phenotype

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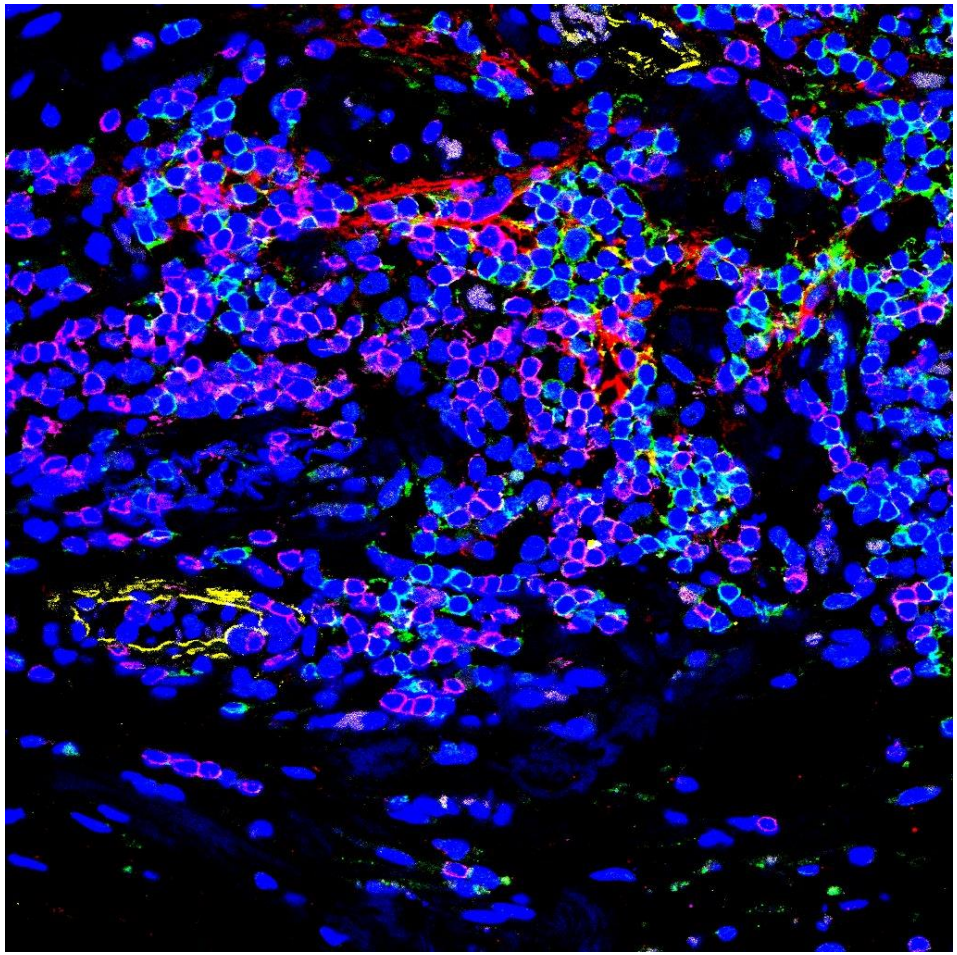
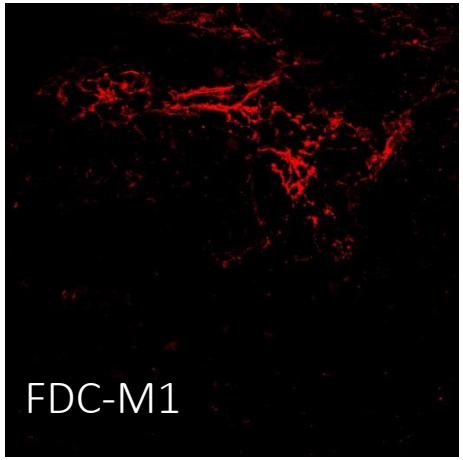
Schlößer et al American Journal of Transplantation 2016

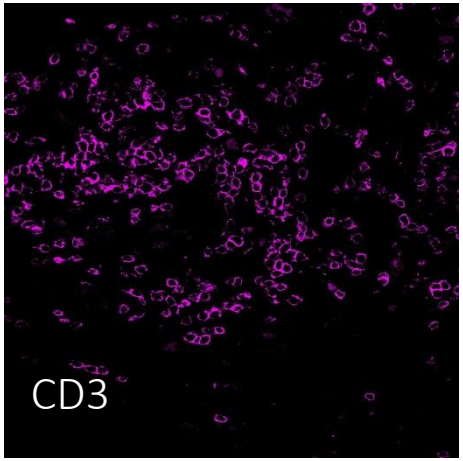
- kidney biopsies 20 each:
  - Stable graft function
  - TCMR
  - ABMR
- Confocale fluorescence microscopy, 6-channels
- Banff classification

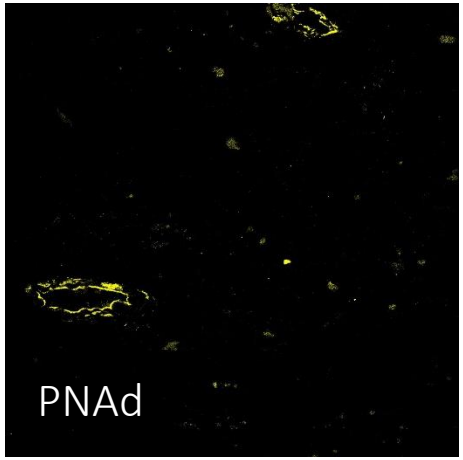
	goat	rat	rabbit	mouse
TLS Composition	CD19	PNAd	CD3	FDC-M1
TLS Function #1	CD19	AID	CD86	CD38
TLS Function #2	CD19	CD3	Ki67	FoxP3
TLS Formation	Lyve1	CD209	LTbR	CD31

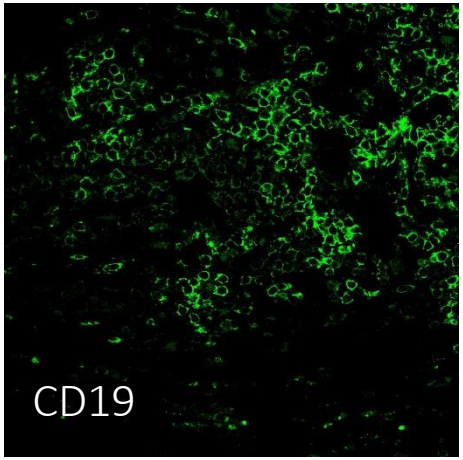
TLS Composition







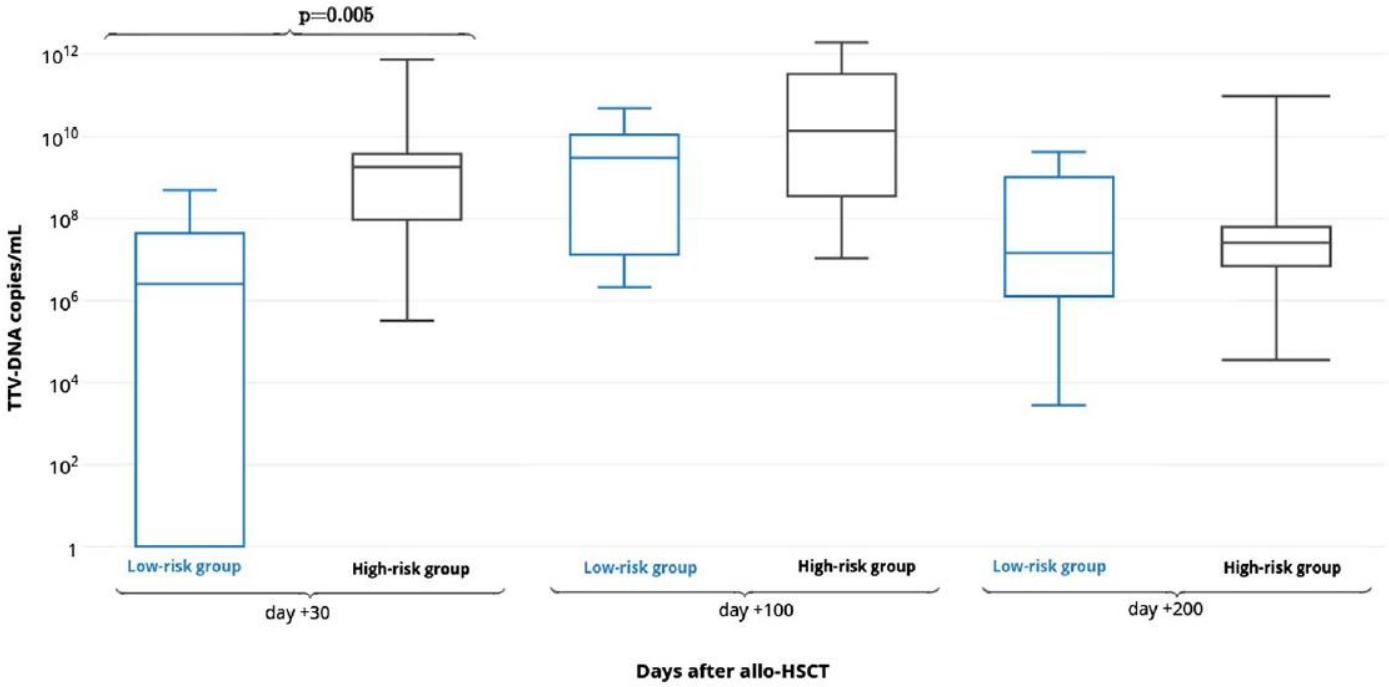




Dynamics of Torque Teno virus viremia could predict risk of complications after allogeneic hematopoietic stem cell transplantation

Ramona Gilles[1] · Marco Herling[2] · Udo Holtick[2] · Eva Heger[1] · Sabine Awerkiew[1] · Irina Fish[1] · Konstantin Höller[1] · Saleta Sierra[1] · Elena Knops[1] · Rolf Kaiser[1] · Christof Scheid[2] · Veronica Di Cristanziano[1]

Med Microbiol Immunol (2017) 206:355–362



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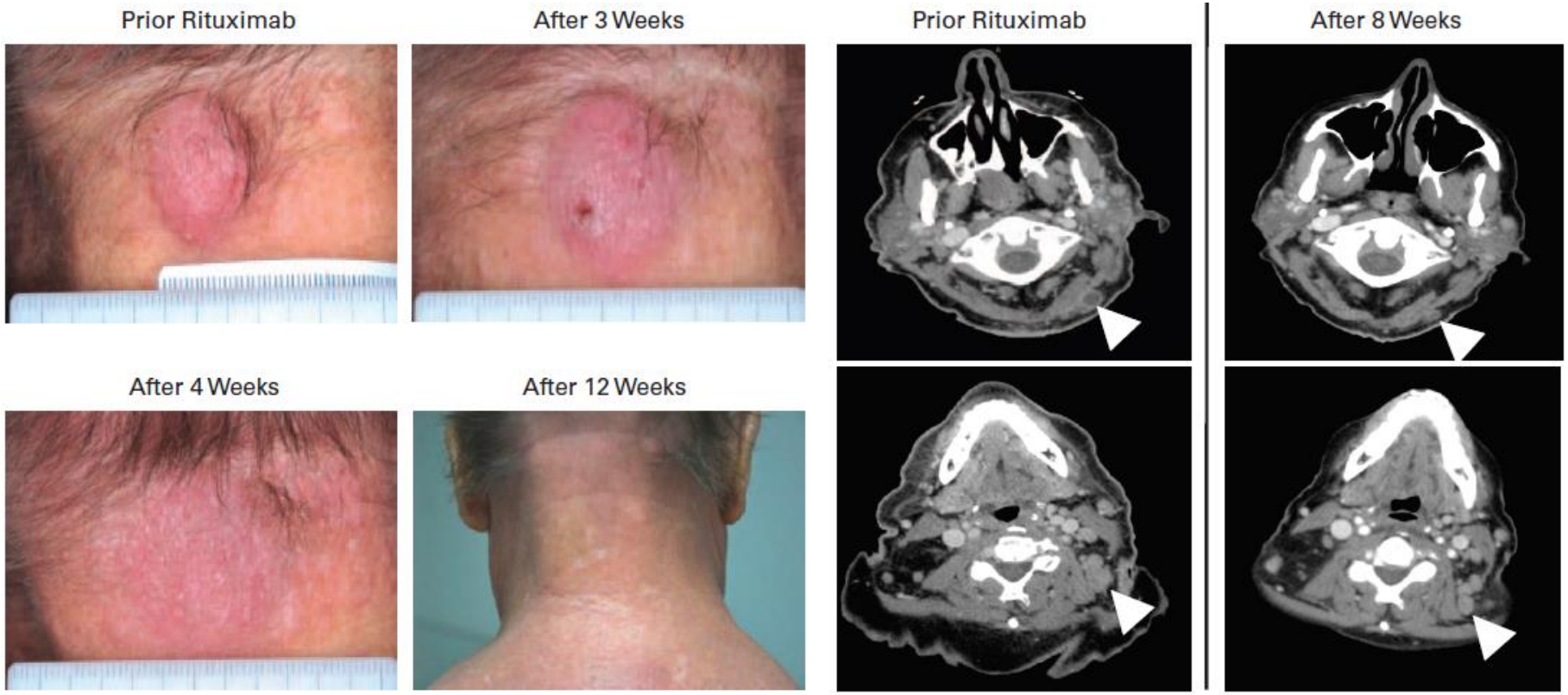
Targeting Tumor-Infiltrating B Cells in Cutaneous T-Cell Lymphoma



Theurich S, JCO 2016

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Conclusions (2)

- B-cell exert important regulatory effects besides antibody production
- still looking for a phenotype, that allows a routine clinical monitoring
- therapeutic interventions not yet possible today
- oncology and infectious disease research programs with synergistic questions

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